Gas-Liquid Chromatographic Test for Honey Adulteration by High Fructose Corn Sirup

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A gas-liquid chromatographic (GLC) method has been developed to detect the addition to honey of high fructose corn sirup (HFCS). Samples are derivatized directly with trimethylsilane, cholestane is added as an internal standard, and the levels of maltose (includes other minor disaccharides) and isomaltose are determined after chromatography on OV-17. Domestic and imported honey samples (115) contained 2.00% maltose and 0.71% isomaltose. HFCS samples (21) contained 1.50% maltose, and 2.09% isomaltose. A discriminatory equation was developed (D = 2.73 − 5.35 (isomaltose/maltose)) and, when applied to the data for these samples and 37 adulterated samples, 81.4% of authentic honey samples and 78.4% of samples known to be adulterated with HFCS were correctly classified.

Bound enzyme technology has resulted in the production of a new industrial sweetener, high fructose corn sirup (HFCS). This is a low-cost, highly refined sirup whose solids typically include about 50% glucose, 42% fructose, and small amounts of higher saccharides (1). Honey, because it is more complex and in limited supply, is a likely target for adulteration by HFCS. We attempted to develop methods to detect the illegal (undeclared) addition of HFCS to honey.

Recently we reported (2) the results of a collaborative study demonstrating that the presence of corn products in honey can be unequivocally shown by measurements of the stable carbon isotope ratio; this method has been adopted as official first action. Detection is possible because the ratio $^{13}C/^{12}C$ in domestic and imported honey is sufficiently consistent (3, 4) and different from that of HFCS (2). Regulatory agencies require rapid screening procedures and often do not possess the proper instrumentation to conduct stable carbon isotope ratio analysis.

This paper provides a rapid screening method for the detection of HFCS in honey. It is based on the difference between the ratios of the disaccharides maltose and isomaltose in honey and HFCS.

Experimental

Apparatus

Gas-liquid chromatograph.—F&M Model 810 equipped with flame ionization detector and 14 ft × ⅛ in. id stainless steel column packed with 3% OV-17 (Applied Science Laboratories, Inc., State College, PA) on 100–200 mesh Gas-Chrom Q (Applied Science Laboratories, Inc.). Operating conditions: injection port 300°C, detector 300°C, column 240°C, immediately programmed at 2°/min; helium carrier flow 50 ml/min, hydrogen flow 10 ml/min, and air flow 400 ml/min.

Reagents

(a) *Solvents.*—Pyridine and hexamethyldisilizane (HMDS, Applied Science Laboratories, Inc.) reagent grade; trifluoroacetic acid (TFA, Pierce Chemical Co., Rockford, IL) sequanal grade.

(b) *Sugars.*—Maltose.H_2O, Grade II or better (Sigma Chemical Co., St. Louis, MO) and isomaltose (ICN Pharmaceuticals, Plainview, NJ).

(c) *Cholestane.*—Internal standard (Gold Label, Aldrich Chemical Co., Milwaukee, WI).

Honey and HFCS Samples

United States honey samples were obtained by solicitation of honey producers, and a certificate of authenticity accompanied each. Nearly 500 samples were collected, representing the 1974 and 1975 honey crop. Imported honey samples were selected from those submitted by 3 United States honey importers. Samples from these that were chosen for analysis had earlier been subjected to stable carbon isotope ratio analysis (3, 4), and details of their selection are reported elsewhere (3). The 37 samples described as known to be adulterated were earlier characterized as such by stable carbon isotope ratio analysis. HFCS samples were received from: Archer Daniels Midland Co., Cedar Rapids, IA; CPC International, Englewood Cliffs, NJ; Clinton Corn Processing Co., Clinton, IA; A. E. Staley Mfg. Co., Decatur, IL;

[1] Retired.

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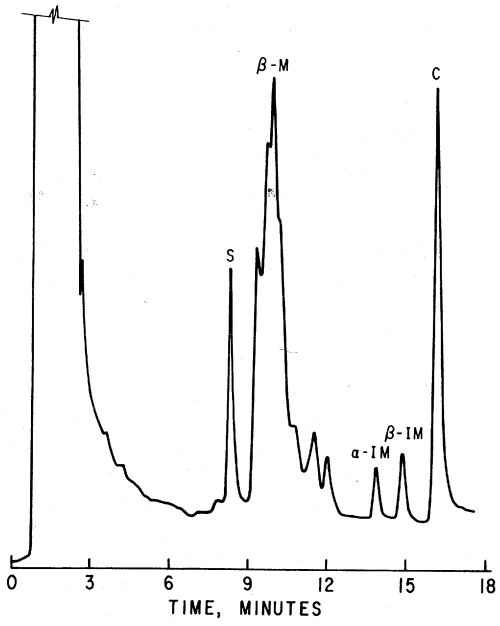

FIG. 1—Gas chromatogram of typical honey: S, sucrose; M, β-maltose; α-IM, α-isomaltose; β-IM, β-isomaltose; C, cholestane.

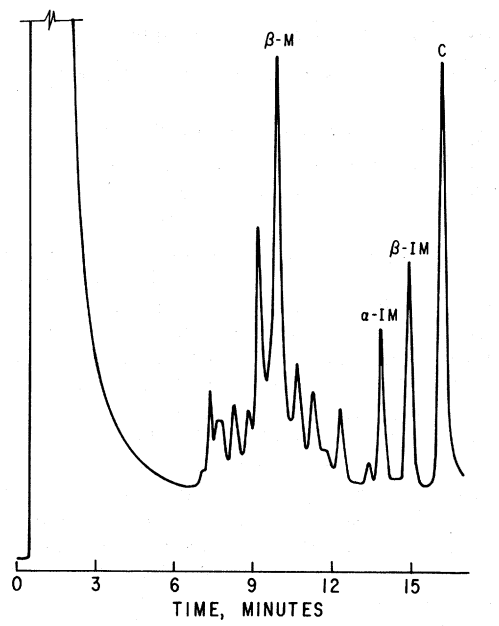

FIG. 2—Gas chromatogram of typical high fructose corn sirup sample: M, β-maltose; α-IM, α-isomaltose; β-IM, β-isomaltose; C, cholestane.

American Maize Products Co., Hammond, IN; Car-Mi Inc., Dayton, OH; and Amstar Corp., Dimmitt, TX. The 21 samples analyzed in this paper include samples from each of these suppliers.

Derivatization Procedure

The procedure used was essentially as described by Brobst and Lott (5). About 60 mg honey or sirup was accurately weighed into a 5 ml screw-top vial. To that was added 1.00 ml pyridine solution containing 1.00 mg cholestane internal standard. After the sample was dissolved, 0.90 ml HMDS was added and mixed; then 0.10 ml TFA was added dropwise and carefully. The sample was shaken 30 sec, and then allowed to stand 15 min with occasional shaking. A homogenous, clear solution resulted; 5.0 μl was withdrawn and injected onto the GLC column. No special precautions were needed for storage of derivatized samples, which were stable for several weeks.

Determination of Maltose and Isomaltose

Typical chromatograms of honey, HFCS, and a mixture of sucrose, maltose (which includes other minor disaccharides), isomaltose, and cholestane are shown in Figs 1, 2, and 3, respectively. Retention times of the standards are: sucrose, 8.4 min; α-maltose, 9.3 min; β-maltose, 10.0 min; α-isomaltose, 13.9 min; β-isomaltose, 14.9 min; and

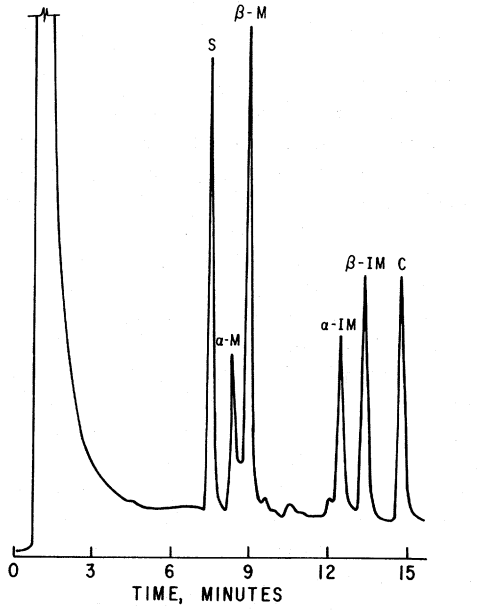

FIG. 3—Gas chromatogram of standard sugars and internal standard: S, sucrose; α-M, α-maltose; β-M, β-maltose; α-IM, α-isomaltose; β-IM, β-isomaltose; C, cholestane.

cholestane, 16.2 min. Maltose and isomaltose levels were calculated on the basis of relative response factors (K) as follows:

$$K = \text{mg cholestane} \times \text{peak height sugar} / \text{mg sugar} \times \text{peak height cholestane}$$

$$\% \text{ sugar} = \text{mg cholestane in sample} \times \text{peak height sugar} \times 100 / K \times \text{peak height cholestane} \times \text{mg sample}$$

Calculations were made from peak heights of the β-anomers of maltose and isomaltose; isomaltose/maltose ratios greater than 0.51 indicate the presence of HFCS. Levels determined for maltose actually included other minor honey and HFCS disaccharides.

Results and Discussion

Numerous methods are available for preparing trimethylsilyl derivatives of carbohydrates for GLC analysis, and those applicable in the presence of moisture were tested. One, in which reducing sugars are converted to oximes and then silylated, had previously been shown to provide chromatograms in which single peaks result and the tautomeric forms of some reducing sugars are eliminated (6). Honey, however, along with the major sugars glucose and fructose, contains at least 10 disaccharides (7), and some of them gave as many as 3 peaks, indicating much less than quantitative conversion to the open-chain oxime derivatives. The GLC profiles in the disaccharide region for honeys derivatized as oxime trimethylsilylates were more complex than the simpler direct procedure chosen for this method.

The derivatized anomers of isomaltose are well separated by GLC from all other honey and high fructose corn sirup disaccharides, but some honey disaccharides have retention times close to that of standard maltose, preventing baseline separation. As a result, maltose levels reported in this paper actually include other disaccharides, but this does not reduce the efficacy of this screening procedure. Figures 1, 2, and 3 are chromatograms of typical honey and HFCS samples and a mixture of standards. The predominant sugars of honey and HFCS are glucose and fructose, which are eluted with the derivatization solvents. The complexity in the disaccharide region of both honey and HFCS is apparent, as is the utility of cholestane as an internal standard in disaccharide analysis. Sucrose(s) is indicated in Figs 1 and 3, and this is the first honey disaccharide eluted.

This is the first example of cholestane being used as an internal standard for sugar analysis, and it satisfies all the criteria for a good internal standard, being a stable hydrocarbon and having a retention time close to that of the sugars being determined.

It was established that the α- and β-anomers of maltose and isomaltose are in equilibrium when honey and HFCS samples are derivatized, as the α:β peak height ratios in the samples were the same as these ratios in the equilibrated standard sugars. So quantitation can be based on the peak height of either anomer, and the β-anomer for both maltose and isomaltose was chosen here because of its greater magnitude.

Table 1 demonstrates the repeatability of triplicate analysis of 4 synthetically prepared mixtures of honey and HFCS. The amount of sample taken for analysis can vary, and the derivatized solutions of sugars are stable for several months at room temperature. Also, the levels of maltose and isomaltose do not change significantly when honey and HFCS samples are stored for extended periods of time before derivatization.

Table 2 summarizes the determinations of maltose, isomaltose, and isomaltose/maltose ratios for 80 domestic honey samples, 35 im-

Table 1. Repeatability of analysis and effects of sample size and time of derivatized mixture storage on maltose,[a] isomaltose, and isomaltose/maltose ratios

Sample	Wt, g	Days of storage	M, %	IM, %	R
1	70.7	0	1.73	0.90	0.52
	45.2	0	1.73	0.85	0.49
	45.2	230	1.71	0.91	0.53
2	52.4	0	1.28	1.04	0.81
	72.3	0	1.33	1.06	0.80
	72.3	230	1.36	1.14	0.84
3	45.6	0	1.36	0.95	0.70
	68.7	0	1.51	0.95	0.63
	68.7	230	1.65	1.20	0.73
4	84.0	0	2.05	0.60	0.29
	42.2	0	1.80	0.58	0.32
	42.2	230	1.84	0.59	0.32

[a] Including other minor disaccharides.

Reference to brand or firm name does not constitute endorsement by the U.S. Department of Agriculture over others of a similar nature not mentioned.

Table 2. Determinations of maltose,[a] isomaltose, and isomaltose/maltose ratios for 80 domestic honey samples, 35 imported honey samples, and 21 HFCS samples

Statistic	Domestic honey			Imported honey			HFCS		
	M	IM	R	M	IM	R	M	IM	R
Mean	1.93	0.64	0.34	2.17	0.87	0.39	0.72	1.50	2.09
Std dev.	0.51	0.37	0.23	0.53	0.50	0.17	0.26	0.82	1.12
Coeff. of var., %	26.4	57.8	67.6	24.4	57.5	43.2	36.1	54.7	53.6

[a] Including other minor disaccharides.

ported honey samples, and 21 samples of HFCS. The honey samples analyzed contain levels of maltose that exceed isomaltose, while the reverse is found for HFCS samples.

Discriminant analysis was applied to 161 samples, including 124 pure honeys and 37 samples that were determined by carbon isotope ratio analysis to be mixtures of honey and HFCS. Various combinations of maltose, isomaltose, and the ratio (R) were considered as possible discriminators. The most useful discriminatory equation is: $D = 2.73–5.35\ (R)$. The sample is classified as adulterated when $D<0$ ($R>0.51$), and as honey when $D>0$ ($R<0.51$). This equation performed as follows: 23 of 124 honey samples were classified as adulterated and 8 of 37 adulterated samples were classified as honey.

This analysis, used in conjunction with another method developed in our laboratory (I. Kushnir (1978) Eastern Regional Research Center, Philadelphia, PA 19118), will enable laboratories not possessing an isotope ratio mass spectrometer to rapidly screen honey samples for confirmatory isotope ratio testing. This method is now being subjected to collaborative study.

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